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(71) Applicant: NOVACEA, INC. [US/US]; 601 Gateway Boulevard, Suite 450, South San Francisco, CA 94080 (US).

(71) Applicants and
(72) Inventors: CHEN, Andrew-Xian [US/US]; 4646 Bryson Terrace, San Diego, CA 92130 (US). FAN, Jun [CN/US]; 10566 Giffin Way, San Diego, CA 92126 (US). YU, Xi-Yun [CN/US]; 11441 Creekstone Lane, San Diego, CA 92128 (US). WHITEHOUSE, Martha, J. [US/US]; 151 Upper Terrace, San Francisco, CA 94117 (US).

(74) Agents: ESMOND, Robert, W. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., 1100 New York Avenue, N.W., Suite 600, Washington, DC 20005-3934 (US).

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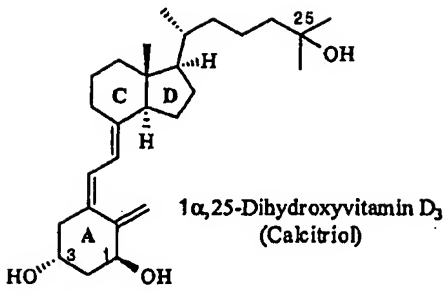
(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING ACTIVE VITAMIN D COMPOUNDS

(57) Abstract: Disclosed are pharmaceutical compositions comprising an active vitamin D compound in emulsion pre-concentrate formulations, as well as emulsions and sub-micron droplet emulsions produced therefrom. The compositions comprise a lipophilic phase component, one or more surfactants, and an active vitamin D compound. The compositions may optionally further comprise a hydrophilic phase component.



1α,25-Dihydroxyvitamin D_3
(Calcitriol)

1α,25-Dihydroxy vitamin D_3 is converted to 1α,24,25-trihydroxy-D_3 by a mitochondrial P450 known as CYP 24 (Bell, N.H., (1998) *J. Bone Miner. Res.* **13**, 350- 35211). CYP 24 is induced by 1α,25-dihydroxy-D_3 and is found in the kidney as well as other vitamin D target tissues such as the parathyroid cells, keratinocytes, osteoblasts, and enteroctyes (Jones, G., Strugnell, S., and DeLuca, H. (1998) *Physiol. Rev.* **78**, 1193-1231). 1α,25-Dihydroxy vitamin D_3 (1,25-D3) has an important role in the antiproliferative and growth regulatory effects on normal and neoplastic cells (for e.g. prostate cancer cells). Clinical use of 1,25-D3 analogs as effective drugs requires antiproliferative and pro-differentiating activities. There is a continuing need for synthetic analogs of 1α,25-dihydroxy vitamin D_3 that selectively exhibit desirable pharmacological activities but do not exhibit hypercalcemic and other undesirable activities.

SUMMARY OF THE INVENTION

Novel 16-ene-25-oxime and 16-ene-25-oxime ether analogs of 1α,25-dihydroxy vitamin D_3 have been prepared that show selective inhibition of the enzyme CYP24, anti-proliferative activity and are low-calcemic.

PHARMACEUTICAL COMPOSITIONS COMPRISING ACTIVE VITAMIN D COMPOUNDS

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to novel pharmaceutical compositions comprising an active vitamin D compound, wherein the pharmaceutical compositions are emulsion pre-concentrates. The invention also relates to emulsions and sub-micron droplet emulsions produced upon dilution of the emulsion pre-concentrates with an aqueous solution.

Background Art

[0002] Vitamin D is a fat soluble vitamin which is essential as a positive regulator of calcium homeostasis. (*See* Harrison's Principles of Internal Medicine: Part Eleven, "Disorders of Bone and Mineral Metabolism," Chapter 335, pp. 1860-1865, E. Braunwald *et al.*, (eds.), McGraw-Hill, New York (1987)). The active form of vitamin D is 1α,25-dihydroxyvitamin D_3, also known as calcitriol. Specific nuclear receptors for active vitamin D compounds have been discovered in cells from diverse organs not involved in calcium homeostasis. (Miller *et al.*, *Cancer Res. 52*:515-520 (1992)). In addition to influencing calcium homeostasis, active vitamin D compounds have been implicated in osteogenesis, modulation of immune response, modulation of the process of insulin secretion by the pancreatic B cell, muscle cell function, and the differentiation and growth of epidermal and hematopoietic tissues.

[0003] Moreover, there have been many reports demonstrating the utility of active vitamin D compounds in the treatment of cancer. For example, it has been shown that certain vitamin D compounds and analogues possess potent antileukemic activity by virtue of inducing the differentiation of malignant cells (specifically, leukemic cells) to non-malignant macrophages (monocytes) and are useful in the treatment of leukemia. (Suda *et al.*, U.S. Patent No. 4,391,802; Partridge *et al.*, U.S. Patent No. 4,594,340). Antiproliferative and differentiating

actions of calcitriol and other vitamin D_3 analogues have also been reported with respect to the treatment of prostate cancer. (Bishop *et al.*, U.S. Patent No. 5,795,882). Active vitamin D compounds have also been implicated in the treatment of skin cancer (Chida *et al.*, *Cancer Research 45*:5426-5430 (1985)), colon cancer (Disman *et al.*, *Cancer Research 47*:21-25 (1987)), and lung cancer (Sato *et al.*, *Tohoku J. Exp. Med. 138*:445-446 (1982)). Other reports suggesting important therapeutic uses of active vitamin D compounds are summarized in Rodriguez *et al.*, U.S. Patent No. 6,034,079.

[0004] Although the administration of active vitamin D compounds may result in substantial therapeutic benefits, the treatment of cancer and other diseases with such compounds is limited by the effects these compounds have on calcium metabolism. At the levels required *in vivo* for effective use as anti-proliferative agents, active vitamin D compounds can induce markedly elevated and potentially dangerous blood calcium levels by virtue of their inherent calcemic activity. That is, the clinical use of calcitriol and other active vitamin D compounds as anti-proliferative agents is precluded, or severely limited, by the risk of hypercalcemia.

[0005] It has been shown that the problem of systemic hypercalcemia can be overcome by "pulse-dose" administration of a sufficient dose of an active vitamin D compound such that an anti-proliferative effect is observed while avoiding the development of severe hypercalcemia. (WO 99/49870). According to WO 99/49870, the active vitamin D compound may be administered no more than every three days, for example, once a week at a dose of at least 0.12 μg/kg per day (8.4 μg in a 70 kg person). Pharmaceutical compositions used in the pulse-dose regimen of WO 99/49870 comprise 5-100 μg of active vitamin D compound and may be administered in the form for oral, intravenous, intramuscular, topical, transdermal, sublingual, intranasal, intratumoral or other preparations.

[0006] ROCALTROL is the trade name of a calcitriol formulation sold by Roche Laboratories. ROCALTROL is available in the form of capsules containing 0.25 and 0.5 μg calcitriol and as an oral solution containing 1 μg/mL of calcitriol. All dosage forms contain butylated hydroxyanisole (BHA) and butylated

hydroxytoluene (BHT) as antioxidants. The capsules also contain a fractionated triglyceride of coconut oil and the oral solution contains a fractionated triglyceride of palm seed oil. (Physician's Desk Reference, 54th Edition, pp 2649-2651, Medical Economics Company, Inc., Montvale, NJ (2000)).

[0007] It is known that calcitriol is light-sensitive and is especially prone to oxidation. Moreover, calcitriol and other active vitamin D compounds are lipophilic, meaning that they are soluble in lipids and some organic solvents, while being substantially insoluble or only sparsely soluble in water. Because of the lipophilic nature of active vitamin D compounds, the dispersion of such compounds in aqueous solutions, such as the gastric fluids of the stomach, is significantly limited. Accordingly, the pharmacokinetic parameters of active vitamin D compound formulations heretofore described in the art are sub-optimal for use with high dose pulse administration regimens. In addition, the active vitamin D compound formulations that are currently available tend to exhibit substantial variability of absorption in the small intestine. Moreover, for oral administration, the relationship between dosage and blood concentration that is observed with most active vitamin D compound formulations is not linear; that is, the quantity of compound absorbed into the blood stream does not correlate with the amount of compound that is administered in a given dose, especially at higher dosage levels.

[0008] Thus, there is a need for improved pharmaceutical compositions comprising active vitamin D compounds, particularly in the context of pulse-dose treatment regimens that are designed to provide anti-proliferative (*e.g.*, anti-cancer) benefits while avoiding the consequence of hypercalcemia. In particular, a need exists in the art for a pharmaceutical composition comprising an active vitamin D compound that remains stable over prolonged periods of time, even at elevated temperatures, while at the same time exhibiting improved pharmacokinetic parameters for the active vitamin D compound, and reduced variability in absorption, when administered to a patient.

BRIEF SUMMARY OF THE INVENTION

[0009] The present invention overcomes the disadvantages heretofore encountered in the art by providing pharmaceutical compositions comprising active vitamin D compounds in emulsion pre-concentrate formulations. The pharmaceutical compositions of the present invention are an advance over the prior art in that they provide a dosage form of active vitamin D compounds, such as calcitriol, in a sufficiently high concentration to permit convenient use, stability and rapid dispersion in solution, and yet meet the required criteria in terms of pharmacokinetic parameters, especially in the context of pulse-dosing administration regimens. More specifically, in a preferred embodiment, the pharmaceutical compositions of the present invention exhibit a C_{max} that is at least 1.5 to two times greater than the C_{max} that is observed with ROCALTROL, and a shorter T_{max} than that which is observed with ROCALTROL.

[0010] The emulsion pre-concentrates of the present invention are non-aqueous formulations for an active vitamin D compound that are capable of providing a pharmaceutically acceptable emulsion, upon contact with water or other aqueous solution.

[0011] According to one aspect of the invention, pharmaceutical compositions are provided comprising (a) a lipophilic phase component, (b) one or more surfactants, and (c) an active vitamin D compound; wherein said composition is an emulsion pre-concentrate, which upon dilution with water in a water to composition ratio of about 1:1 or more of water forms an emulsion having an absorbance of greater than 0.3 at 400 nm. According to this aspect of the invention, the pharmaceutical compositions may further comprise a hydrophilic phase component.

[0012] According to another aspect of the invention, a pharmaceutical emulsion composition is provided comprising water and an emulsion pre-concentrate, said emulsion pre-concentrate comprising (a) a lipophilic phase component, (b) one or more surfactants, and (c) an active vitamin D compound, and optionally, a hydrophobic phase component.

[0013] The emulsions produced from the emulsion pre-concentrates of the present invention (upon dilution with water) include both emulsions as conventionally understood by those of ordinary skill in the art (*i.e.*, a dispersion of an organic phase in water), as well as "sub-micron droplet emulsions" (*i.e.*, dispersions of an organic phase in water wherein the average diameter of the dispersion particles is less than 1000 nm.)

[0014] According to another aspect of the invention, methods are provided for the preparation of emulsion pre-concentrates comprising active vitamin D compounds. The methods encompassed within this aspect of the invention comprise bringing an active vitamin D compound, *e.g.*, calcitriol, into intimate admixture with a lipophilic phase component and with one or more surfactants, and optionally, with a hydrophilic phase component.

[0015] In yet another aspect of the invention, methods are provided for the treatment and prevention of hyperproliferative diseases such as cancer and psoriasis, said methods comprising administering an active vitamin D compound in an emulsion pre-concentrate formulation to a patient in need thereof. Alternatively, the active vitamin D compound can be administered in an emulsion formulation that is made by diluting an emulsion pre-concentrate of the present invention with an appropriate quantity of water. In a preferred embodiment of this aspect of the invention, the administration of the active vitamin D compound to a patient is accomplished by using, *e.g.*, a pulse dosing regimen. For example, according to this aspect of the invention, an active vitamin D compound in an emulsion pre-concentrate formulation is administered to a patient no more than once every three days at a dose of at least 0.12 μg/kg per day.

BRIEF DESCRIPTIONS OF THE FIGURES

[0016] Fig. 1 is a graphical representation of the mean plasma concentration of calcitriol in dogs versus time following administration of three different formulations of calcitriol at a dose of 1 μg/kg.

[0017] Figs. 2A and 2B are graphical representations of the mean plasma concentration-time curve for calcitriol after escalating doses of semi-solid #3 in male (Fig. 2A) and female (Fig. 2B) dogs.

[0018] Figs. 3A and 3B are graphical representations of the plasma concentration-time curve for calcitriol in male (Fig. 3A) and female (Fig. 3B) dogs after semi-solid #3 dosing.

[0019] Figs. 4A and 4B are graphical representations of the mean serum calcium after increasing doses of semi-solid #3 in male (Fig. 4A) and female (Fig. 4B) dogs.

[0020] Figs. 5A-5C are graphical representations of the plasma calcitriol and serum calcium data following administration of semi-solid #3 in male dogs.

[0021] Fig. 6 is a graphical representation of the mean plasma concentration of calcitriol by dose group in humans following administration of semi-solid #3.

DETAILED DESCRIPTION OF THE INVENTION

[0022] The present invention is directed to pharmaceutical compositions comprising active vitamin D compounds in emulsion pre-concentrate formulations. The compositions of the invention meet or substantially reduce the difficulties associated with active vitamin D compound therapy hitherto encountered in the art including, in particular, undesirable pharmacokinetic parameters of the compound upon administration to a patient.

[0023] It has been found that the compositions of the invention permit the preparation of semi-solid and liquid compositions containing an active vitamin D compound in sufficiently high concentration to permit, *e.g.*, convenient oral

administration, while at the same time achieving improved pharmacokinetic parameters for the active vitamin D compound. For example, as compared to ROCALTROL, the compositions of the present invention exhibit a C_{max} that is at least 1.5 to two times greater than the C_{max} that is observed with ROCALTROL, and a shorter T_{max} than that which is observed with ROCALTROL. Preferably, the pharmaceutical compositions of the present invention provide a C_{max} of at least about 900 pg/mL plasma, more preferably about 900 to about 3000 pg/mL plasma, more preferably about 1500 to about 3000 pg/mL plasma. In addition, the compositions of the invention preferably provide a T_{max} of less than about 6.0 hours, more preferably about 1.0 to about 3.0 hours, more preferably about 1.5 to about 2.0 hours. In addition, the compositions of the invention preferably provide a $T_{1/2}$ of less than about 25 hours, more preferably about 2 to about 10 hours, more preferably about 5 to about 9 hours.

[0024] The term C_{max} is defined as the maximum concentration of active vitamin D compound achieved in the serum following administration of the drug. The term T_{max} is defined as the time at which C_{max} is achieved. The term $T_{1/2}$ is defined as the time required for the concentration of active vitamin D compound in the serum to decrease by half. The disclosed values for pharmacokinetic data apply to the population of recipients of a composition comprising an active vitamin D compound as a whole, not individual recipients. Thus, any individual receiving a composition of the present invention may not necessarily achieve the preferred pharmacokinetic parameters. However, when a composition of the present invention is administered to a sufficiently large population of subjects, the pharmacokinetic parameters will approximately match the values disclosed herein.

[0025] According to one aspect of the present invention, a pharmaceutical composition is provided comprising (a) a lipophilic phase component, (b) one or more surfactants, (c) an active vitamin D compound; wherein said composition is an emulsion pre-concentrate, which upon dilution with water, in a water to composition ratio of about 1:1 or more of said water, forms an emulsion having

an absorbance of greater than 0.3 at 400 nm. The pharmaceutical composition of the invention may further comprise a hydrophilic phase component.

[0026] In another aspect of the invention, a pharmaceutical emulsion composition is provided comprising water (or other aqueous solution) and an emulsion pre-concentrate.

[0027] The term "emulsion pre-concentrate," as used herein, is intended to mean a system capable of providing an emulsion upon contacting with, *e.g.*, water. The term "emulsion," as used herein, is intended to mean a colloidal dispersion comprising water and organic components including hydrophobic (lipophilic) organic components. The term "emulsion" is intended to encompass both conventional emulsions, as understood by those skilled in the art, as well as "sub-micron droplet emulsions," as defined immediately below.

[0028] The term "sub-micron droplet emulsion," as used herein is intended to mean a dispersion comprising water and organic components including hydrophobic (lipophilic) organic components, wherein the droplets or particles formed from the organic components have an average maximum dimension of less than about 1000 nm.

[0029] Sub-micron droplet emulsions are identifiable as possessing one or more of the following characteristics. They are formed spontaneously or substantially spontaneously when their components are brought into contact, that is without substantial energy supply, *e.g.*, in the absence of heating or the use of high shear equipment or other substantial agitation.

[0030] The particles of a sub-micron droplet emulsion may be spherical, though other structures are feasible, e.g. liquid crystals with lamellar, hexagonal or isotropic symmetries. Generally, sub-micron droplet emulsions comprise droplets or particles having a maximum dimension (*e.g.*, average diameter) of between about 50 nm to about 1000 nm, and preferably between about 200 nm to about 300 nm.

[0031] The term "pharmaceutical composition" as used herein is to be understood as defining compositions of which the individual components or ingredients are themselves pharmaceutically acceptable, *e.g.*, where oral administration is

foreseen, acceptable for oral use and, where topical administration is foreseen, topically acceptable.

[0032] The pharmaceutical compositions of the present invention will generally form an emulsion upon dilution with water. The emulsion will form according to the present invention upon the dilution of an emulsion pre-concentrate with water in a water to composition ratio of about 1:1 or more of said water. According to the present invention, the ratio of water to composition can be, *e.g.*, between 1:1 and 5000:1. For example, the ratio of water to composition can be about 1:1, 2:1, 3:1, 4:1, 5:1, 10:1, 200:1, 300:1, 500:1, 1000:1, or 5000:1. The skilled artisan will be able to readily ascertain the particular ratio of water to composition that is appropriate for any given situation or circumstance.

[0033] According to the present invention, upon dilution of said emulsion pre-concentrate with water, an emulsion will form having an absorbance of greater than 0.3 at 400 nm. The absorbance at 400 nm of the emulsions formed upon 1:100 dilution of the emulsion pre-concentrates of the present invention can be, *e.g.*, between 0.3 and 4.0. For example, the absorbance at 400 nm can be, *e.g.*, about 0.4, 0.5, 0.6, 1.0, 1.2, 1.6, 2.0, 2.2, 2.4, 2.5, 3.0, or 4.0. Methods for determining the absorbance of a liquid solution are well known by those in the art. The skilled artisan will be able to ascertain and adjust the relative proportions of the ingredients of the emulsions pre-concentrates of the invention in order to obtain, upon dilution with water, an emulsion having any particular absorbance encompassed within the scope of the invention.

[0034] The pharmaceutical compositions of the present invention can be, *e.g.*, in a semi-solid formulation or in a liquid formulation. Semi-solid formulations of the present invention can be any semi-solid formulation known by those of ordinary skill in the art, including, *e.g.*, gels, pastes, creams and ointments.

[0035] The pharmaceutical compositions of the present invention comprise a lipophilic phase component. Suitable components for use as lipophilic phase components include any pharmaceutically acceptable solvent which is non-miscible with water. Such solvents will appropriately be devoid or substantially devoid of surfactant function.

[0036] The lipophilic phase component may comprise mono-, di- or triglycerides. Mono-, di- and triglycerides that may be used within the scope of the invention include those that are derived from C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20} and C_{22} fatty acids. Exemplary diglycerides include, in particular, diolein, dipalmitolein, and mixed caprylin-caprin diglycerides. Preferred triglycerides include vegetable oils, fish oils, animal fats, hydrogenated vegetable oils, partially hydrogenated vegetable oils, synthetic triglycerides, modified triglycerides, fractionated triglycerides, medium and long-chain triglycerides, structured triglycerides, and mixtures thereof.

[0037] Among the above-listed triglycerides, preferred triglycerides include: almond oil; babassu oil; borage oil; blackcurrant seed oil; canola oil; castor oil; coconut oil; corn oil; cottonseed oil; evening primrose oil; grapeseed oil; groundnut oil; mustard seed oil; olive oil; palm oil; palm kernel oil; peanut oil; rapeseed oil; safflower oil; sesame oil; shark liver oil; soybean oil; sunflower oil; hydrogenated castor oil; hydrogenated coconut oil; hydrogenated palm oil; hydrogenated soybean oil; hydrogenated vegetable oil; hydrogenated cottonseed and castor oil; partially hydrogenated soybean oil; partially soy and cottonseed oil; glyceryl tricaproate; glyceryl tricaprylate; glyceryl tricaprate; glyceryl triundecanoate; glyceryl trilaurate; glyceryl trioleate; glyceryl trilinoleate; glyceryl trilinolenate; glyceryl tricaprylate/caprate; glyceryl tricaprylate/caprate/laurate; glyceryl tricaprylate/caprate/linoleate; and glyceryl tricaprylate/caprate/stearate.

[0038] A preferred triglyceride is the medium chain triglyceride available under the trade name LABRAFAC CC. Other preferred triglycerides include neutral oils, *e.g.*, neutral plant oils, in particular fractionated coconut oils such as known and commercially available under the trade name MIGLYOL, including the products: MIGLYOL 810; MIGLYOL 812; MIGLYOL 818; and CAPTEX 355.

[0039] Also suitable are caprylic-capric acid triglycerides such as known and commercially available under the trade name MYRITOL, including the product MYRITOL 813. Further suitable products of this class are CAPMUL MCT, CAPTEX 200, CAPTEX 300, CAPTEX 800, NEOBEE M5 and MAZOL 1400.

[0040] Especially preferred as lipophilic phase component is the product MIGLYOL 812. (*See* U.S. Patent No. 5,342,625).

[0041] Pharmaceutical compositions of the present invention may further comprise a hydrophilic phase component. The hydrophilic phase component may comprise, *e.g.*, a pharmaceutically acceptable C_{1-5} alkyl or tetrahydrofurfuryl di- or partial-ether of a low molecular weight mono- or poly-oxy-alkanediol. Suitable hydrophilic phase components include, *e.g.*, di- or partial-, especially partial-, -ethers of mono- or poly-, especially mono- or di-, -oxy-alkanediols comprising from 2 to 12, especially 4 carbon atoms. Preferably the mono- or poly-oxy-alkanediol moiety is straight-chained. Exemplary hydrophilic phase components for use in relation to the present invention are those known and commercially available under the trade names TRANSCUTOL and COLYCOFUROL. (*See* U.S. Patent No. 5,342,625).

[0042] In an especially preferred embodiment, the hydrophilic phase component comprises 1,2-propyleneglycol.

[0043] The hydrophilic phase component of the present invention may of course additionally include one or more additional ingredients. Preferably, however, any additional ingredients will comprise materials in which the active vitamin D compound is sufficiently soluble, such that the efficacy of the hydrophilic phase as an active vitamin D compound carrier medium is not materially impaired. Examples of possible additional hydrophilic phase components include lower (*e.g.*, C_{1-5}) alkanols, in particular ethanol.

[0044] Pharmaceutical compositions of the present invention also comprise one or more surfactants. Surfactants that can be used in conjunction with the present invention include hydrophilic or lipophilic surfactants, or mixtures thereof. Especially preferred are non-ionic hydrophilic and non-ionic lipophilic surfactants.

[0045] Suitable hydrophilic surfactants include reaction products of natural or hydrogenated vegetable oils and ethylene glycol, *i.e.* polyoxyethylene glycolated natural or hydrogenated vegetable oils, for example polyoxyethylene glycolated natural or hydrogenated castor oils. Such products may be obtained in known

manner, *e.g.*, by reaction of a natural or hydrogenated castor oil or fractions thereof with ethylene oxide, *e.g.*, in a molar ratio of from about 1:35 to about 1:60, with optional removal of free polyethyleneglycol components from the product, *e.g.*, in accordance with the methods disclosed in German Auslegeschriften 1,182,388 and 1,518,819.

[0046] Suitable hydrophilic surfactants for use in the present pharmaceutical compounds also include polyoxyethylene-sorbitan-fatty acid esters, *e.g.*, mono- and trilauryl, palmityl, stearyl and oleyl esters, *e.g.*, of the type known and commercially available under the trade name TWEEN; including the products:
TWEEN 20 (polyoxyethylene(20)sorbitanmonolaurate),
TWEEN 40 (polyoxyethylene(20)sorbitanmonopalmitate),
TWEEN 60 (polyoxyethylene(20)sorbitanmonostearate),
TWEEN 80 (polyoxyethylene(20)sorbitanmonooleate),
TWEEN 65 (polyoxyethylene(20)sorbitantristearate),
TWEEN 85 (polyoxyethylene(20)sorbitantrioleate),
TWEEN 21 (polyoxyethylene(4)sorbitanmonolaurate),
TWEEN 61 (polyoxyethylene(4)sorbitanmonostearate), and
TWEEN 81 (polyoxyethylene(5)sorbitanmonooleate).

[0047] Especially preferred products of this class for use in the compositions of the invention are the above products TWEEN 40 and TWEEN 80. (*See* Hauer, *et al.*, U.S. Patent No. 5,342,625).

[0048] Also suitable as hydrophilic surfactants for use in the present pharmaceutical compounds are polyoxyethylene alkylethers; polyoxyethylene glycol fatty acid esters, for example polyoxythylene stearic acid esters; polyglycerol fatty acid esters; polyoxyethylene glycerides; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction mixtures of polyols and, *e.g.*, fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; polyoxyethylene-polyoxypropylene co-polymers; polyoxyethylene-polyoxypropylene block co-polymers; dioctylsuccinate, dioctylsodiumsulfosuccinate, di-[2-ethylhexyl]-succinate or sodium lauryl sulfate; phospholipids, in particular lecithins such as, *e.g.*, soya bean lecithins; propylene

glycol mono- and di-fatty acid esters such as, *e.g.*, propylene glycol dicaprylate, propylene glycol dilaurate, propylene glycol hydroxystearate, propylene glycol isostearate, propylene glycol laurate, propylene glycol ricinoleate, propylene glycol stearate, and, especially preferred, propylene glycol caprylic-capric acid diester; and bile salts, *e.g.*, alkali metal salts, for example sodium taurocholate.

[0049] Suitable lipophilic surfactants include alcohols; polyoxyethylene alkylethers; fatty acids; bile acids; glycerol fatty acid esters; acetylated glycerol fatty acid esters; lower alcohol fatty acids esters; polyethylene glycol fatty acids esters; polyethylene glycol glycerol fatty acid esters; polypropylene glycol fatty acid esters; polyoxyethylene glycerides; lactic acid esters of mono/diglycerides; propylene glycol diglycerides; sorbitan fatty acid esters; polyoxyethylene sorbitan fatty acid esters; polyoxyethylene-polyoxypropylene block copolymers; trans-esterified vegetable oils; sterols; sugar esters; sugar ethers; sucroglycerides; polyoxyethylene vegetable oils; polyoxyethylene hydrogenated vegetable oils; reaction mixtures of polyols and at least one member of the group consisting of fatty acids, glycerides, vegetable oils, hydrogenated vegetable oils, and sterols; and mixtures thereof.

[0050] Suitable lipophilic surfactants for use in the present pharmaceutical compounds also include trans-esterification products of natural vegetable oil triglycerides and polyalkylene polyols. Such trans-esterification products are known in the art and may be obtained *e.g.*, in accordance with the general procedures described in U.S. Pat. No. 3,288,824. They include trans-esterification products of various natural (*e.g.*, non-hydrogenated) vegetable oils for example, maize oil, kernel oil, almond oil, ground nut oil, olive oil and palm oil and mixtures thereof with polyethylene glycols, in particular polyethylene glycols having an average molecular weight of from 200 to 800. Preferred are products obtained by trans-esterification of 2 molar parts of a natural vegetable oil triglyceride with one molar part of polyethylene glycol (*e.g.*, having an average molecular weight of from 200 to 800). Various forms of trans-esterification products of the defined class are known and commercially available under the trade name LABRAFIL.

[0051] Additional lipophilic surfactants that are suitable for use with the present pharmaceutical compositions include oil-soluble vitamin derivatives, *e.g.*, tocopherol PEG-1000 succinate ("vitamin E TPGS").

[0052] Also suitable as lipophilic surfactants for use in the present pharmaceutical compounds are mono-, di- and mono/di-glycerides, especially esterification products of caprylic or capric acid with glycerol; sorbitan fatty acid esters; pentaerythritol fatty acid esters and polyalkylene glycol ethers, for example pentaerythrite- -dioleate, -distearate, -monolaurate, -polyglycol ether and -monostearate as well as pentaerythrite-fatty acid esters; monoglycerides, *e.g.*, glycerol monooleate, glycerol monopalmitate and glycerol monostearate; glycerol triacetate or (1,2,3)-triacetin; and sterols and derivatives thereof, for example cholesterols and derivatives thereof, in particular phytosterols, *e.g.*, products comprising sitosterol, campesterol or stigmasterol, and ethylene oxide adducts thereof, for example soya sterols and derivatives thereof.

[0053] It is understood by those of ordinary skill in the art that several commercial surfactant compositions contain small to moderate amounts of triglycerides, typically as a result of incomplete reaction of a triglyceride starting material in, for example, a trans-esterification reaction. Thus, the surfactants that are suitable for use in the present pharmaceutical compositions include those surfactants that contain a triglyceride. Examples of commercial surfactant compositions containing triglycerides include some members of the surfactant families GELUCIRES, MAISINES, AND IMWITORS. Specific examples of these compounds are GELUCIRE 44/14 (saturated polyglycolized glycerides); GELUCIRE 50/13 (saturated polyglycolized glycerides); GELUCIRE 53/10 (saturated polyglycolized glycerides); GELUCIRE 33/01 (semi-synthetic triglycerides of C_8 -C_{18} saturated fatty acids); GELUCIRE 39/01 (semi-synthetic glycerides); other GELUCIRE, such as 37/06, 43/01, 35/10, 37/02, 46/07, 48/09, 50/02, 62/05, etc.; MAISINE 35-I (linoleic glycerides); and IMWITOR 742 (caprylic/capric glycerides). (*See* U.S. Patent No. 6,267,985).

[0054] Still other commercial surfactant compositions having significant triglyceride content are known to those skilled in the art. It should be appreciated

The amounts of such additives can be readily determined by one skilled in the art, according to the particular properties desired.

[0057] The additive may also comprise a thickening agent. Suitable thickening agents may be of those known and employed in the art, including, *e.g.*, pharmaceutically acceptable polymeric materials and inorganic thickening agents. Exemplary thickening agents for use in the present pharmaceutical compositions include polyacrylate and polyacrylate co-polymer resins, for example poly-acrylic acid and poly-acrylic acid/methacrylic acid resins; celluloses and cellulose derivatives including: alkyl celluloses, *e.g.*, methyl-, ethyl- and propyl-celluloses; hydroxyalkyl-celluloses, *e.g.*, hydroxypropyl-celluloses and hydroxypropylalkyl-celluloses such as hydroxypropyl-methyl-celluloses; acylated celluloses, *e.g.*, cellulose-acetates, cellulose-acetatephthallates, cellulose-acetatesuccinates and hydroxypropylmethyl-cellulose phthallates; and salts thereof such as sodium-carboxymethyl-celluloses; polyvinylpyrrolidones, including for example poly-N-vinylpyrrolidones and vinylpyrrolidone co-polymers such as vinylpyrrolidone-vinylacetate co-polymers; polyvinyl resins, *e.g.*, including polyvinylacetates and alcohols, as well as other polymeric materials including gum traganth, gum arabicum, alginates, *e.g.*, alginic acid, and salts thereof, *e.g.*, sodium alginates; and inorganic thickening agents such as atapulgite, bentonite and silicates including hydrophilic silicon dioxide products, *e.g.*, alkylated (for example methylated) silica gels, in particular colloidal silicon dioxide products.

[0058] Such thickening agents as described above may be included, *e.g.*, to provide a sustained release effect. However, where oral administration is intended, the use of thickening agents as aforesaid will generally not be required and is generally less preferred. Use of thickening agents is, on the other hand, indicated, *e.g.*, where topical application is foreseen.

[0059] Compositions in accordance with the present invention may be employed for administration in any appropriate manner, *e.g.*, orally, *e.g.*, in unit dosage form, for example in a solution, in hard or soft encapsulated form including gelatin encapsulated form, *e.g.*, parenterally or topically, *e.g.*, for application to the skin, for example in the form of a cream, paste, lotion, gel, ointment, poultice,

that such compositions, which contain triglycerides as well as surfactants, may be suitable to provide all or part of the lipophilic phase component of the of the present invention, as well as all or part of the surfactants.

[0055] The pharmaceutical compositions of the present invention also comprise an active vitamin D compound. The term "active vitamin D compound," as used herein, is intended to refer to vitamin D which has been hydroxylated in at least the carbon-1 position of the A ring, *e.g.*, 1α-hydroxyvitamin D3. The preferred active vitamin D compound in relation to the composition of the present invention is 1α,25- hydroxyvitamin D_3, also known as calcitriol. A large number of other active vitamin D compounds are known and can be used in the practice of the invention. Examples include 1α-hydroxy derivatives with a 17 side chain greater in length than the cholesterol or ergosterol side chains (*see* U.S. Patent No. 4,717,721); cyclopentano-vitamin D analogs (*see* U.S. Patent No. 4,851,401); vitamin D_3 analogues with alkynyl, alkenyl, and alkanyl side chains (*see* U.S. Patent Nos. 4, 866,048 and 5,145,846); trihydroxycalciferol (*see* U.S. Patent No. 5,120,722); fluoro-cholecalciferol compounds (*see* U.S. Patent No.5,547,947); methyl substituted vitamin D (*see* U.S. Patent No. 5,446,035); 23-oxa-derivatives (*see* U.S. Patent No. 5,411,949); 19-nor-vitamin D compounds (*see* U.S. Patent No. 5,237,110); and hydroxylated 24-homo-vitamin D derivatives (*see* U.S. Patent No. 4,857,518). Particular examples include ROCALTROL (Roche Laboratories); CALCIJEX injectable calcitriol; investigational drugs from Leo Pharmaceuticals including EB 1089 (24a,26a,27a-trihomo-22,24-diene-1αa,25-$(OH)_2$-D_3, KH 1060 (20-epi-22-oxa-24a,26a,27a-trihomo-1α,25-$(OH)_2$-D_3), Seocalcitol, MC 1288 (1,25-$(OH)_2$-20-epi-D_3) and MC 903 (calcipotriol, 1α24s-$(OH)_2$-22-ene-26,27-dehydro-D_3); Roche Pharmaceutical drugs that include 1,25-$(OH)_2$-16-ene-D_3, 1,25-$(OH)_2$-16-ene-23-yne-D_3, and 25-$(OH)_2$-16-ene-23-yne-D_3; Chugai Pharmaceuticals 22-oxacalcitriol (22-oxa-1α,25-$(OH)_2$-D_3; 1α-(OH)-D_5 from the University of Illinois; and drugs from the Institute of Medical Chemistry-Schering AG that include ZK 161422 (20-methyl-1,25-$(OH)_2$-D_3) and ZK 157202 (20-methyl-23-ene-1,25-$(OH)_2$-D_3); 1α-(OH)-D_2; 1α-(OH)-D_3 and 1α-(OH)-D_4. Additional examples include 1α,25-$(OH)_2$-26,27-d_6-D_3; 1α,25-$(OH)_2$-

22-ene-D_3; 1α,25-$(OH)_2$-D_3; 1α,25-$(OH)_2$-D_2; 1α,25-$(OH)_2$-D_4; 1α,24,25-$(OH)_3$-D_3; 1α,24,25-$(OH)_3$-D_2; 1α,24,25-$(OH)_3$-D_4; 1α-(OH)-25-FD_3; 1α-(OH)-25-FD_4; 1α-(OH)-25-FD_2; 1α,24-$(OH)_2$-D_4; 1α,24-$(OH)_2$-D_3; 1α,24-$(OH)_2$-D_2; 1α,24-$(OH)_2$-25-FD_4; 1α,24-$(OH)_2$-25-FD_3; 1α,24-$(OH)_2$-25-FD_2; 1α,25-$(OH)_2$-26,27-F_6-22-ene-D_3; 1α,25-$(OH)_2$-26,27-F_6-D_3; 1α,25S-$(OH)_2$-26-F_3-D_3; 1α,25-$(OH)_2$-24-F_2-D_3; 1α,25S,26-$(OH)_2$-22-ene-D_3; 1α,25R,26-$(OH)_2$-22-ene-D_3; 1α,25-$(OH)_2$-D_2; 1α,25-$(OH)_2$-24-epi-D_3; 1α,25-$(OH)_2$-23-yne-D_3; 1α,25-$(OH)_2$-24R-F-D_3; 1α,25S,26-$(OH)_2$-D_3; 1α,24R-$(OH)_2$-25F-D_3; 1α,25-$(OH)_2$-26,27-F_6-23-yne-D_3; 1α,25R-$(OH)_2$-26-F_3-D_3; 1α,25,28-$(OH)_3$-D_2; 1α,25-$(OH)_2$-16-ene-23-yne-D_3; 1α,24R,25-$(OH)_3$-D_3; 1α,25-$(OH)_2$-26,27-F_6-23-ene-D_3; 1α,25R-$(OH)_2$-22-ene-26-F_3-D_3; 1α,25S-$(OH)_2$-22-ene-26-F_3-D_3; 1α,25R-$(OH)_2$-D_3-26,26,26-d_3; 1α,25S-$(OH)_2$-D_3-26,26,26-d_3; and 1α,25R-$(OH)_2$-22-ene-D_3-26,26,26-d_3. Additional examples can be found in WO 99/49870. *See also, e.g.*, U.S. Patent Nos. 5,457,217, 5,447,924, 5,446,034, 5,414,098, 5,403,940, 5,384,313, 5,374,629, 5,373,004, 5,371,249, 5,430,196, 5,260,290, 5,393,749, 5,395,830, 5,250,523, 5,247,104, 5,397,775, 5,194,431, 5,281,731, 5,254,538, 5,232,836, 5,185,150, 5,321,018, 5,086,191, 5,036,061, 5,030,772, 5,246,925, 4,973,584, 5,354,744, 4,927,815, 4,804,502, 4,857,518, 4,851,401, 4,851,400, 4,847,012, 4,755,329, 4,940,700, 4,619,920, 4,594,192, 4,588,716, 4,564,474, 4,552,698, 4,588,528, 4,719,204, 4,719,205, 4,689,180, 4,505,906, 4,769,181, 4,502,991, 4,481,198, 4,448,726, 4,448,721, 4,428,946, 4,411,833, 4,367,177, 4,336,193, 4,360,472, 4,360,471, 4,307,231, 4,307,025, 4,358,406, 4,305,880, 4,279,826, and 4,248,791.

[0056] The pharmaceutical compositions of the present invention may further comprise one or more additives. Additives that are well known in the art include, *e.g.*, detackifiers, anti-foaming agents, buffering agents, antioxidants (*e.g.*, ascorbyl palmitate, butyl hydroxy anisole (BHA), butyl hydroxy toluene (BHT) and tocopherols, *e.g.*, α-tocopherol (vitamin E)), preservatives, chelating agents, viscomodulators, tonicifiers, flavorants, colorants odorants, opacifiers, suspending agents, binders, fillers, plasticizers, lubricants, and mixtures thereof.

cataplasm, plaster, dermal patch or the like, or for ophthalmic application, for example in the form of an eye-drop, -lotion or -gel formulation. Readily flowable forms, for example solutions and emulsions, may also be employed *e.g.*, for intralesional injection, or may be administered rectally, *e.g.*, as an enema.

[0060] When the composition of the present invention is formulated in unit dosage form, the active vitamin D compound will preferably be present in an amount of between 10 and 75 μg per unit dose. More preferably, the amount of active vitamin D compound per unit dose will be about 10 μg, 15 μg, 20 μg, 25 μg, 30 μg, 35 μg, 40 μg, 45 μg, 50 μg, 55 μg, 60 μg, 65 μg, 70 μg, or 75 μg.

[0061] When the unit dosage form of the composition is a capsule, the total quantity of ingredients present in the capsule is preferably about 10-1000 μL. More preferably, the total quantity of ingredients present in the capsule is about 100-300 μL.

[0062] The relative proportion of ingredients in the compositions of the invention will, of course, vary considerably depending on the particular type of composition concerned. The relative proportions will also vary depending on the particular function of ingredients in the composition. The relative proportions will also vary depending on the particular ingredients employed and the desired physical characteristics of the product composition, *e.g.*, in the case of a composition for topical use, whether this is to be a free flowing liquid or a paste. Determination of workable proportions in any particular instance will generally be within the capability of a person of ordinary skill in the art. All indicated proportions and relative weight ranges described below are accordingly to be understood as being indicative of preferred or individually inventive teachings only and not as not limiting the invention in its broadest aspect.

[0063] The lipophilic phase component of the invention will suitably be present in an amount of from about 30% to about 90% by weight based upon the total weight of the composition. Preferably, the lipophilic phase component is present in an amount of from about 50% to about 85% by weight based upon the total weight of the composition.

[0064] The surfactant or surfactants of the invention will suitably be present in an amount of from about 1% to 50% by weight based upon the total weight of the composition. Preferably, the surfactant(s) is present in an amount of from about 5% to about 40% by weight based upon the total weight of the composition.

[0065] The amount of active vitamin D compound in compositions of the invention will of course vary, *e.g.*, depending on the intended route of administration and to what extent other components are present. In general, however, the active vitamin D compound of the invention will suitably be present in an amount of from about 0.005% to 20% by weight based upon the total weight of the composition. Preferably, the active vitamin D compound is present in an amount of from about 0.01% to 15% by weight based upon the total weight of the composition.

[0066] The hydrophilic phase component of the invention will suitably be present in an amount of from about 2% to about 20% by weight based upon the total weight of the composition. Preferably, the hydrophilic phase component is present in an amount of from about 5% to 15% by weight based upon the total weight of the composition.

[0067] The pharmaceutical composition of the invention may be in a semisolid formulation. Semisolid formulations within the scope of the invention may comprise, *e.g.*, a lipophilic phase component present in an amount of from about 60% to about 80% by weight based upon the total weight of the composition, a surfactant present in an amount of from about 5% to about 35% by weight based upon the total weight of the composition, and an active vitamin D compound present in an amount of from about 0.01% to about 15% by weight based upon the total weight of the composition.

[0068] The pharmaceutical compositions of the invention may be in a liquid formulation. Liquid formulations within the scope of the invention may comprise, *e.g.*, a lipophilic phase component present in an amount of from about 50% to about 60% by weight based upon the total weight of the composition, a surfactant present in an amount of from about 4% to about 25% by weight based upon the total weight of the composition, an active vitamin D compound present

in an amount of from about 0.01% to about 15% by weight based upon the total weight of the composition, and a hydrophilic phase component present in an amount of from about 5% to about 10% by weight based upon the total weight of the composition.

[0069] In addition to the foregoing the present invention also provides a process for the production of a pharmaceutical composition as hereinbefore defined, which process comprises bringing the individual components thereof into intimate admixture and, when required, compounding the obtained composition in unit dosage form, for example filling said composition into gelatin, *e.g.*, soft or hard gelatin, capsules, or non-gelatin capsules.

[0070] In a more particular embodiment, the invention provides a process for the preparation of a pharmaceutical composition, which process comprises bringing an active vitamin D compound, *e.g.*, calcitriol, into close admixture with a lipophilic phase component and a surfactant as hereinbefore defined, the relative proportion of the lipophilic phase component and the surfactant being selected relative to the quantity of active vitamin D compound employed, such that an emulsion pre-concentrate is obtained.

[0071] The present invention also provides methods for the treatment and prevention of hyperproliferative diseases such as cancer and psoriasis, said methods comprising administering an active vitamin D compound in an emulsion pre-concentrate formulation to a patient in need thereof. Alternatively, the active vitamin D compound can be administered in an emulsion formulation that is made by diluting an emulsion pre-concentrate of the present invention with an appropriate quantity of water.

[0072] Cancers which can be treated with the formulations of the invention include any cancer treatable by an active vitamin D compound. Such cancers include without limitation cancers of the prostate, breast, colon, lung, head and neck, pancreas, endometrium, bladder, cervix, ovaries, squamous cell carcinoma, renal cell carcinoma, myeloid and lymphocytic leukemia, lymphoma, medullary thyroid carcinoma, melanoma, multiple myeloma, retinoblastoma and sarcomas of the soft tissues and bone.

[0073] Preferably, the cancers are treated according to the pulse dose protocols disclosed in WO 99/49870. In this embodiment, the formulations are administered no more than once every three days, more preferably, no more than once a week, more preferably, no more than once every ten days. Preferably, about 5 to about 100 μg of calcitriol, more preferably, about 10 to 60 μg, more preferably, about 40-50 μg of calcitriol, or an equivalent amount of another active vitamin D compound, is administered to an animal in need thereof.

[0074] Animals which may be treated according to the present invention include all animals which may benefit from administration of the formulations of the present invention. Such animals include humans, pets such as dogs and cats, and veterinary animals such as cows, pigs, sheep, goats and the like.

[0075] The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in clinical therapy and which are obvious to those skilled in the art are within the spirit and scope of the invention.

EXAMPLES

EXAMPLE 1

Relative Chemical Compatibility of Calcitriol With Selected Components

[0076] In this example, the relative chemical compatibility of calcitriol with selected lipophilic, hydrophilic and surfactant components was evaluated by measuring the percent recovery of intact calcitriol after storage at 40°C and 60°C. Calcitriol recovery was determined based on analyses of high-pressure liquid chromatography (HPLC). The results are presented in Table 1.

TABLE 1: Percent Recovery of Calcitriol Formulated in Selected Components

Component	Excipient	Time	% Recovery at 40°C	% Recovery at 60°C
Lipophilic	Corn oil	0	100.00	100.00
		3 days	93.77	104.80
		7 days	90.27	91.50
		14 days	89.89	86.46
	Soybean oil	0	100.00	100.00
		3 days	96.44	94.56
		7 days	98.46	98.57
		14 days	96.66	93.15
	Sunflower oil	0	100.00	100.00
		3 days	99.10	99.33
		7 days	102.77	102.93
		14 days	96.56	88.79
	Vitamin E	0	100.00	100.00
		3 days	128.56	160.79
		7 days	0.00	0.00
		14 days	102.29	65.02
	Miglyol 812	0	100.00	100.00
		3 days	98.23	97.01
		7 days	99.31	96.78
		14 days	99.17	99.48
	Miglyol 812, 0.02% BHA/BHT	0	100.00	100.00
		3 days	98.41	97.83
		7 days	97.43	98.17
		14 days	98.72	102.15
	Captex 200	0	100.00	100.00
		3 days	99.20	97.28
		7 days	100.14	97.68
		14 days	108.83	101.15
	Labrafac CC	0	100.00	100.00
		3 days	98.60	95.84
		7 days	100.05	99.51
		14 days	101.37	100.24

Component	Excipient	Time	% Recovery at 40°C	% Recovery at 60°C
Hydrophilic	PEG 300	0	100.00	100.00
		3 days	78.22	18.95
		7 days	52.68	4.61
		14 days	10.09	1.84
	Propylene Glycol	0	100.00	100.00
		3 days	97.56	99.71
		7 days	101.73	108.47
		14 days	105.83	138.22
Surfactant	Cremophor ELP	0	100.00	100.00
		3 days	82.61	66.28
		7 days	62.86	60.90
		14 days	51.90	59.92
	Cremophor RH 40 25%	0	100.00	100.00
		3 days	105.30	91.91
		7 days	92.10	78.30
		14 days	96.88	87.95
	in Miglyol 812			
	Polysorbate 80	0	100.00	100.00
		3 days	87.94	67.43
		7 days	87.29	71.71
		14 days	60.52	66.08
	GELUCIRE 44/14 25%	0	100.00	100.00
		3 days	98.70	107.68
		7 days	101.55	83.06
		14 days	100.96	98.11
	in Miglyol 812			
	Vitamin E TPGS 25%	0	100.00	100.00
		3 days	101.15	97.26
		7 days	101.26	98.74
		14 days	103.61	100.15
	in Miglyol 812			
	Labrifil M	0	100.00	100.00
		3 days	98.46	95.19
		7 days	99.45	95.64
		14 days	100.30	78.97
	Poloxamer 188 25% in Miglyol 812	0	100.00	100.00
		3 days	116.42	76.47
		7 days	126.39	116.67
		14 days	126.79	83.30

[0077] The recovery data suggest that the most compatible components are Miglyol 812 (with or without BHT and BHA), Labrafac CC and Captex 200 in the lipophilic component group, propylene glycol in the hydrophilic group, and vitamin E TPGS and GELUCIRE 44/14 in the surfactant group.

EXAMPLE 2

Stability of Liquid and Semi-Solid Calcitriol Formulations

I. Introduction

[0078] In this Example, the stability of the active vitamin D compound calcitriol was measured in nine different formulations (four liquid formulations and five semisolid formulations).

II. Preparation of Calcitriol Formulations

A. Liquid Formulations

[0079] Four liquid calcitriol formulations (L1-L4) were prepared containing the ingredients listed in Table 2. The final formulation contains 0.208 mg calcitriol per gram of liquid formulation.

TABLE 2: Composition of Liquid Calcitriol Formulations

Ingredient	L1	L2	L3	L4
Calcitriol	0.0208	0.0208	0.0208	0.0208
Miglyol 812	56.0	62.0	0	0
Captex 200	0	0	55.0	0
Labrafac CC	0	0	0	55.0
Vitamin-E TPGS	15.0	24.0	22.0	20.0
Labrifil M	23.0	4.0	14.0	15.0
1,2-propylene glycol	6.0	10.0	9.0	10.0
BHT	0.05	0.05	0.05	0.05
BHA	0.05	0.05	0.05	0.05

Amounts shown are in grams.

B. Semi-Solid Formulations

[0080] Five semi-solid calcitriol formulations (SS1-SS5) were prepared containing the ingredients listed in Table 3. The final formulation contains 0.208 mg calcitriol per gram of semi-solid formulation.

TABLE 3: Composition of Semi-Solid Calcitriol Formulations

Ingredient	SS1	SS2	SS3	SS4	SS5
Calcitriol	0.0208	0.0208	0.0208	0.0208	0.0208
Miglyol 812	80.0	0	65.0	0	79.0
Captex 200	0	82.0	0	60.0	0
Labrafac CC	0	0	0	0	12.0
Vitamin-E TPGS	20.0	18.0	5.0	5.0	9.0
Labrifil M	0	0	0	0	0
Gelucire 44/14	0	0	30.0	35.0	0
BHT	0.05	0.05	0.05	0.05	0.05
BHA	0.05	0.05	0.05	0.05	0.05

Amounts shown are in grams.

C. Method of Making the Liquid and Semi-Solid Calcitriol Formulations

1. Preparation of Vehicles

[0081] One hundred gram quantities of the four liquid calcitriol formulations (L1-L4) and the five semi-solid calcitriol formulations (SS1-SS5) listed in Tables 2 and 3, respectively, were prepared as follows.

[0082] The listed ingredients, except for calcitriol, were combined in a suitable glass container and mixed until homogeneous. Vitamin E TPGS and GELUCIRE 44/14 were heated and homogenized at 60°C prior to weighing and adding into the formulation.

2. Preparation of Active Formulations

[0083] The semi-solid vehicles were heated and homogenized at ≤ 60°C. Under subdued light, 12 ± 1 mg of calcitriol was weighed out into separate glass bottles with screw caps, one bottle for each formulation. (Calcitriol is light-sensitive;

subdued light/red light should be used when working with calcitriol/calcitriol formulations.) The exact weight was recorded to 0.1 mg. The caps were then placed on the bottles as soon as the calcitriol had been placed into the bottles. Next, the amount of each vehicle required to bring the concentration to 0.208 mg/g was calculated using the following formula:

$$C_w/0.208 = \text{required weight of vehicle}$$

Where C_w = weight of calcitriol, in mg, and

0.208 = final concentration of calcitriol (mg/g).

[0084] Finally, the appropriate amount of each vehicle was added to the respective bottle containing the calcitriol. The formulations were heated (≤ 60°C) while being mixed to dissolve the calcitriol.

III. Stability of Calcitriol Formulations

[0085] The nine calcitriol formulations (L1-L4 and SS1-SS5) were analyzed for stability of the calcitriol component at three different temperatures. Sample of the nine formulations were each placed at 25°C, 40°C, and 60°C. Samples from all three temperatures for all nine formulations were analyzed by HPLC after 1, 2 and 3 weeks. In addition, samples from the 60°C experiment were analyzed by HPLC after 9 weeks. The percent of the initial calcitriol concentration remaining at each time point was determined for each sample and is reported in Table 4 (liquid formulations) and Table 5 (semi-solid formulations).

TABLE 4: Stability of Liquid Formulations

		Recovery* of Calcitriol (%)			
Formulation	Temp.	Week 1	Week 2	Week 3	Week 9
Liquid #1	25°C	99.3	98.6	99.7	ND
	40°C	103.2	100.4	100.2	ND
	60°C	99.4	98.4	98.4	91.7
Liquid #2	25°C	98.1	95.2	97.7	ND
	40°C	98.0	97.1	99.2	ND
	60°C	97.1	95.6	96.7	93.1
Liquid #3	25°C	99.7	99.2	102.3	ND
	40°C	100.1	99.9	100.7	ND
	60°C	98.3	98.7	98.4	90.5
Liquid #4	25°C	98.4	97.7	98.0	ND
	40°C	100.0	101.0	100.8	ND
	60°C	98.5	97.5	99.0	86.1

* Percent of time zero concentration.

TABLE 5: Stability of Semi-Solid Formulations

		Recovery* of Calcitriol (%)			
Formulation	Temp.	Week 1	Week 2	Week 3	Week 9
Semi-Solid #1	25°C	98.5	98.9	99.8	ND
	40°C	99.6	99.0	98.2	ND
	60°C	97.9	97.2	96.3	104.6
Semi-Solid #2	25°C	100.0	99.6	100.4	ND
	40°C	98.7	99.6	98.7	ND
	60°C	97.2	98.0	98.6	100.0
Semi-Solid #3	25°C	101.2	98.9	100.4	ND
	40°C	100.0	98.7	98.8	ND
	60°C	98.3	97.6	98.4	97.1
Semi-Solid #4	25°C	100.2	99.0	99.6	ND
	40°C	98.4	99.2	98.5	ND
	60°C	96.8	97.7	97.7	103.4
Semi-Solid #5	25°C	98.8	99.2	98.9	ND
	40°C	99.0	97.1	96.8	ND
	60°C	96.8	96.7	96.0	97.7

* Percent of time zero concentration.

[0086] As illustrated by Tables 4 and 5, calcitriol remained relatively stable with very little degradation in all of the formulations (liquid and semi-solid) analyzed.

EXAMPLE 3

Appearance and UV/Visible Absorption Study of Calcitriol Formulations

[0087] Calcitriol formulations L1 and SS3 were prepared prior to this study and stored at room temperature protected from light. Table 6 below shows the quantities of ingredients used to prepare the formulations.

TABLE 6: Composition of Calcitriol Formulations Used for Absorption Analysis

Ingredient	Liquid #1	Semi-Solid #3
Calcitriol	0.0131	0.0136
Vitamin-E TPGS	9.45	3.27
Miglyol 812	35.28	42.51
Labrifil M	14.49	0
Gelucire 44/14	0	19.62
1,2-propylene glycol	3.78	0
BHA	0.03	0.03
BHT	0.03	0.03

Amounts shown are in grams.

[0088] The formulations were warmed to 55°C prior to use. Both formulations (liquid #1 and semi-solid #3) were mixed well with a vortex mixer and appeared as clear liquids. Each calcitriol formulation (≈250 μL) was added to a 25 mL volumetric flask. The exact weights added were 249.8 mg for Liquid-1 and 252.6 mg for semi-solid #3. Upon contact with the glass, the semi-solid-3 formulation became solidified. Deionized water was then added to the 25 mL mark and the solutions were mixed with a vortex mixer until uniform. The appearance was observed at this point and the absorbance of the resulting mixtures at 400 nm was determined by UV/visible spectrophotometry. Deionized water was used as a blank and the measurements were taken at 400 nm. Each sample was measured 10 times over a period of 10 minutes. The results are summarized in Table 7. Both formulations formed were white and opaque.

TABLE 7: Absorption Readings of the Formulations at 400 nm

Measurement	Liquid #1	Semi-Solid #3
1	2.4831	1.6253
2	2.5258	1.6290
3	2.5411	1.6309
4	2.5569	1.6328
5	2.5411	1.6328
6	2.5258	1.6347
7	2.5569	1.6328
8	2.5111	1.6366
9	2.5111	1.6366
10	2.5411	1.6328
Average	2.5294	1.6324
RSD%	0.91	0.21

EXAMPLE 4

Diameter of Emulsion Droplets Formed From the Liquid and Semi-Solid Formulation Vehicles (without calcitriol)

[0089] In this example, the average diameter of emulsion droplets was measured after dilution of the liquid (L1-L4) and semi-solid (SS1-SS5) emulsion pre-concentrate vehicles (not containing calcitriol) with simulated gastric fluid (SGF) lacking enzyme. The average diameter of the droplets was determined based on light scattering measurements. The appearance of the pre-concentrates and the resulting emulsions, determined by visual inspection, was also noted. The results are summarized in Table 8.

TABLE 8: Diameter of Emulsion Droplets Formed From Emulsion Pre-Concentrate Vehicles (without calcitriol)

Formulation	Appearance of emulsion pre-concentrate	pre-concentrate: SGF ratio	Ave. hydro-dynamic diameter*	Appearance of emulsion
L1	Clear liquid	1:1600	237	opaque
L2	Clear liquid	1:1600	281	opaque
L3	Clear liquid	1:1600	175	opaque
L4	Clear liquid	1:1600	273	opaque
SS1	Semi-solid	1:2000	305	opaque
SS2	Semi-solid	1:2000	259	opaque
SS3	Semi-solid	1:2000	243	opaque
SS4	Semi-solid	1:2000	253	opaque
SS5	Semi-solid	1:2000	267	opaque

*(Zaverage in nanometer)

[0090] From the results presented above, it is concluded that the droplets (particles) formed from the emulsion preconcentrate formulations were of sub-micron droplet size despite having an opaque appearance.

EXAMPLE 5

Diameter of Emulsion Droplets Formed From Liquid and Semi-Solid Calcitriol Formulation

[0091] In this example, the average diameter of emulsion droplets was measured after dilution of the liquid #1 (L1) and semi-solid #3 (SS3) emulsion pre-concentrates in simulated gastric fluid (SGF) without enzyme. The formulations used in this example contained calcitriol at a concentration of 0.2 mg calcitriol/g of formulation. The diameter of the droplets was determined based on light scattering measurements. The appearance of the resulting emulsions, determined by visual inspection, was also noted. The results are summarized in Table 9.

TABLE 9: Diameter of Emulsion Droplets Formed From Emulsion Pre-Concentrate Formulations Containing Calcitriol

Formulation	pre-concentrate: SGF ratio	Ave. hydro-dynamic diameter*	Appearance of emulsion
L1	1:1600	257	opaque
SS3	1:2000	263	opaque

*(Zaverage in nanometer)

EXAMPLE 6

In Vitro Dispersion of Calcitriol From Emulsion Pre-Concentrates

[0092] In this Example, the extent of calcitriol dispersion in various formulations in gelatin capsules was determined. A single capsule containing 250 mg of a calcitriol formulation in a size-2 gelatin capsule (each capsule containing 0.2mg calcitriol/g formulation) was added to 200 mL of simulated gastric fluid (SGF) without enzyme at 37°C and was mixed by a paddle at 200 RPM. Samples were then filtered through a 5 μm filter and analyzed for calcitriol concentration at 30, 60, 90, and 120 minutes by HPLC. The results are shown in Table 10.

TABLE 10: Percent Calcitriol Obtained in Filtrate After Dispersion in SGF and Filtration Through a 5 μm Filter

Formulation	30 min.	60 min.	90 min.	120 min.
Liquid #1	106	103	86	68
Semi-Solid #3	109	99	73	53
Comparison Formulation#	0	0	0	0

#The Comparison Formulation contained calcitriol at 0.2 mg/g dissolved in Miglyol 812 with 0.05% BHA and 0.05% BHT. This formulation is similar to the ROCALTROL formulation available from Roche Laboratories.

[0093] As this Example illustrates, the dispersion of calcitriol in simulated gastric fluid from capsules containing either the L1 or the SS3 formulations was much more extensive than that which was observed with capsules containing the

Comparison Formulation (which is similar to the ROCALTROL formulation available from Roche Laboratories).

EXAMPLE 7

Plasma Concentrations and Pharmacokinetics of Calcitriol in Dogs

[0094] A pharmacokinetics study in dogs compared the plasma levels of calcitriol after administration of 1.0 μg/kg using 3 different formulations: ROCALTROL, a liquid formulation (liquid #1, and a semi-solid formulation (semi-solid #3). Four dogs received 1.0 μg/kg orally of ROCALTROL, the semi-solid formulation, or the liquid formulation. When dogs were used for more than one formulation a minimum 7-day washout period separated dosing with each formulation.

[0095] Blood samples were obtained pre-dose, and 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, and 48 hours post-dose for analysis of calcitriol levels. Blood samples for clinical chemistry were obtained pre-dose, and at 24 and 48 hours post-dose for the ROCALTROL group; samples were obtained pre-dose, and at 4, 24, 48, 72, 96, and 120 hours for the semi-solid and liquid formulations. Samples were analyzed for calcitriol by radioimmunoassay and subjected to pharmacokinetics analyses.

[0096] Plasma concentrations of calcitriol over time for the three formulations are shown graphically in Figure 1.

[0097] A summary of the pharmacokinetics of calcitriol as one of three different formulations at a common dose of 1.0 μg/kg is presented in Tables 11-14.

TABLE 11: Summary of Calcitriol Parameters in Dogs

Parameter	ROCALTROL		Semi-Solid #3		Liquid #1	
	Mean	SD	Mean	SD	Mean	SD
C_{max}, pg/mL	717.4	51.5	2066.6	552.5	2164.4	253.9
T_{max}[a], h	3.0	(2-6)	2.0	(1-2)	1.5	(1-2)
$AUC_{(0-t)}$, pg·h/mL	11988.0	3804.7	12351.7	1624.9	14997.4	3531.7
$T_{1/2}$[b], h	25.1	11.1	4.8	1.2	7.8	3.5

[a] Expressed as median and range

[b] Expressed as harmonic mean and pseudo SD based on jackknife variance

TABLE 12: Plasma Concentration (pg/mL) and Pharmacokinetic Parameters of Calcitriol in Dog Following a Single 1 µg/kg Administration of ROCALTROL

Parameter	Time, h	Dog 101	Dog 102	Dog 103	Dog 104	Mean	SD
	0.0	BQL	BQL	BQL	BQL	0	0
	0.5	488.2	304.8	182.7	BQL	243.9	205.4
	1.0	478.2	634.8	500.7	555.7	542.4	69.7
	2.0	518.2	700.8	749.7	765.7	683.6	113.7
	4.0	494.2	658.8	750.7	745.7	662.4	119.8
	6.0	652.2	566.8	496.7	523.7	559.9	68.0
	8.0	381.2	366.8	418.7	381.7	387.1	22.2
	10.0	313.2	212.8	165.7	158.7	212.6	71.2
	12.0	190.2	186.8	189.7	171.7	184.6	8.7
	24.0	78.2	78.8	69.7	97.7	81.1	11.8
	36.0	63.2	83.8	80.7	67.7	73.9	10.0
	48.0	66.2	47.8	45.7	52.7	53.1	9.2
C_{max}, pg/mL		652.2	700.8	750.7	765.7	717.4	51.5
T_{max}[a], h		6.0	2.0	4.0	2.0	3.0	(2-6)
$AUC_{(0-t)}$, pg·h/mL		17693.6	10094.5	9976.2	10187.5	11988.0	3804.7
$T_{1/2}$[b], h		100.4	18.8	20.2	21.3	25.1	11.1

[a] Expressed as median and range

[b] Expressed as harmonic mean and pseudo SD based on jackknife variance

Bold type - used to calculate λ

TABLE 13: Plasma Concentration (pg/mL) and Pharmacokinetic Parameters of Calcitriol in Dog Following a Single 1 μg/kg Administration of Semi-solid #3 Formulation

Parameter	Time, h	Dog 101	Dog 102	Dog 103	Dog 104	Mean	SD
	0.0	BQL	BQL	BQL	BQL	0	0
	0.5	198.1	11.0	BQL	BQL	52.3	97.4
	1.0	1208.1	2246.0	1128.7	503.4	1271.6	722.0
	2.0	1255.1	2110.0	2269.7	2495.4	2032.6	541.9
	4.0	902.1	1371.0	1095.7	1437.4	1201.6	248.5
	6.0	603.1	1039.0	932.7	1112.4	921.8	224.9
	8.0	815.1	441.0	593.7	848.4	674.6	192.4
	10.0	253.1	489.0	285.7	305.4	333.3	106.0
	12.0	213.1	295.0	184.7	170.4	215.8	55.7
	24.0	50.1	37.0	40.7	29.4	39.3	8.6
	36.0	14.1	BQL	BQL	13.6	6.9	8.0
	48.0	BQL	BQL	BQL	BQL	0.0	0.0
C_{max}, pg/mL		1255.1	2246.0	2269.7	2495.4	2066.6	552.5
T_{max}[a], h		2.0	1.0	2.0	2.0	2.0	(1-2)
$AUC_{(0-t)}$, pg·h/mL		10333.8	14012.9	11813.8	13246.4	12351.7	1624.9
$T_{1/2}$[b], h		6.2	3.8	4.1	5.9	4.8	1.2

[a] Expressed as median and range

[b] Expressed as harmonic mean and pseudo SD based on jackknife variance

Bold type - used to calculate λ

TABLE 14: Plasma Concentration (pg/mL) and Pharmacokinetic Parameters of Calcitriol in Dogs Following a Single 1 µg/kg Liquid #1 Formulation

Parameter	Time, h	Dog 105	Dog 106	Dog 107	Dog 108	Mean	SD
	0.0	BQL	BQL	BQL	BQL	0	0
	0.5	BQL	57.6	523.0	350.0	232.7	246.9
	1.0	1283.0	238.6	2266.0	2468.0	1563.9	1024.0
	2.0	2028.0	1895.6	2026.0	2373.0	2080.7	204.5
	4.0	1090.0	892.6	1009.0	1771.0	1190.7	395.3
	6.0	871.0	763.6	730.0	1063.0	856.9	150.0
	8.0	301.0	579.6	374.0	562.0	454.2	138.1
	10.0	421.0	520.6	464.0	517.0	480.7	47.4
	12.0	348.0	290.6	170.0	373.0	295.4	90.4
	24.0	42.0	165.6	62.0	202.0	117.9	78.0
	36.0	49.0	111.6	BQL	79.0	59.9	47.4
	48.0	35.0	15.5	BQL	BQL	12.6	16.6
C_{max}, pg/mL		2028.0	1895.6	2266.0	2468.0	2164.4	253.9
T_{max}[a], h		2.0	2.0	1.0	1.0	1.5	(1-2)
$AUC_{(0-\infty)}$, pg·h/mL		13474.4	14296.3	12101.0	20117.7	14997.4	3531.7
$T_{1/2}$[b], h		10.6	8.5	5.0	10.1	7.8	3.5

[a] Expressed as median and range
[b] Expressed as harmonic mean and pseudo SD based on jackknife variance
Bold type - used to calculate l

[0098] The results of this study show that there were some differences and similarities in the pharmacokinetics between these particular inventive formulations and ROCALTROL as follows:

- C_{max} was approximately three times higher with the liquid and semi-solid formulations than with the ROCALTROL formulation.
- C_{max} was achieved sooner (1 to 2 hours) with the liquid and semi-solid formulations than with the ROCALTROL formulation (2 to 4 hours).
- The overall systemic exposure ($AUC_{0-\infty}$) was comparable with the three formulations, although systemic exposure in the first 24-48 hours was greater with the liquid and semi-solid formulations than with ROCALTROL.

[0099] The foregoing results show that the liquid #1 formulation produces the highest C_{max} and the largest AUC calcitriol values, followed closely by the semi-solid #3 formulation. The ROCALTROL formulation has the lowest C_{max} and AUC values. It appears that the liquid #1 and semi-solid #3 formulations were

absorbed much faster and produced higher plasma concentration during the first twelve hours and a faster rate of elimination.

EXAMPLE 8

Pharmacokinetics of the Semi-Solid #3 Formulation After Escalating Doses

[0100] In this study the pharmacokinetics of the semi-solid formulation after escalating oral doses was studied in dogs. Three male and three female Beagle dogs were dosed orally with single doses of 0.5 μg/kg (all six dogs), 0.1 μg/kg (1 male and 1 female), 5.0 μg/kg (2 males and 2 females), and 10.0 μg/kg (all dogs). After the 10.0 μg/kg dose, 2 dogs per sex were euthanized. The remaining male and female dogs continued on study and received doses of 30.0 μg/kg and 100.0 μg/kg. After each dose the animals were held for a 6-day recovery period.

[0101] Blood samples (approximately 1 mL) were collected from each dog pre-dose and at 0, 2 (in all but the 0.5 μg/kg dose), 4, 8, 24, 48, and 96 hours following dose administration. Samples were analyzed for calcitriol by radioimmunoassay and subjected to pharmacokinetic analyses. Plasma concentrations of calcitriol are shown graphically for males and females in Figs. 2A and 2B.

[0102] After dosing with semi-solid #3, maximum plasma concentrations usually occurred at the two hour sampling timepoint. At doses above 0.1 μg/kg, plasma concentrations appeared to decline at a more rapid rate during the first 8 hours than during the 24 to 96 hour time period.

[0103] At the lowest dose of 0.1 μg/kg, plasma concentrations of calcitriol fell below the limit of quantitation after 24 hours. At 0.5 μg/kg and above, measurable concentrations of calcitriol persisted at the 96 hour sampling timepoint. There did not appear to be any remarkable differences between the male and the female dogs.

[0104] Pharmacokinetic parameters for semi-solid #3 at doses ranging from 0.1 to 100.0 μg/kg are summarized in Table 15.

Table 15: Pharmacokinetics of Calcitriol After Escalating Doses of Calcitriol (Semi-solid #3)

Dose (µg/kg)	0.1		0.5		5.0	
Gender	Male	Female	Male	Female	Male	Female
N	1	1	3	3	2	2
C_{max} (pg/mL)	566	473	1257	1431	17753	18346
Tmax (hr)	2.0	2.0	4.0	4.0	2.0	2.0
AUC_{0-24} (pg·hr/mL)	4311	2654	11431	15598	104,027	107,452
AUC_{0-48} (pg·hr/mL)	4311	2654	13584	19330	125,408	126,746
$AUC_{0-\infty}$ (pg·hr/mL)	4916	2718	15062	21644	200,283	160,681
$T_{1/2}$ (hr)	4.2	2.7	17.1	14.2	67.6	36.8

Dose (µg/kg)	10.0		30.0		100.0	
Gender	Male	Female	Male	Female	Male	Female
N	3	3	1	1	1	1
C_{max} (pg/mL)	23858	32336	53005	115,896	238,619	211,631
Tmax (hr)	2.7	2.0	2.0	2.0	2.0	2.0
AUC_{0-24} (pg·hr/mL)	183,981	203,857	311,841	567,717	1,165,988	1,089,831
AUC_{0-48} (pg·hr/mL)	223,977	240,483	370,713	641,469	1,381,424	1,256,007
$AUC_{0-\infty}$ (pg·hr/mL)	388,600	345,936	531,303	854,841	1,874,997	1,731,873
$T_{1/2}$ (hr)	77.7	56.0	56.3	58.2	45.3	53.7

[0105] These pharmacokinetic results indicate the following:

- The systemic exposure of calcitriol appeared to be fairly linear throughout the tested dose range of 0.1 to 100.0 µg/kg. No saturation of absorption was observed.
- The half-life of calcitriol appeared to be dose-dependent. Formulations having a half life of greater than 24 hours are less suitable for high dose pulse administration.

- Weekly dosing with semi-solid #3 at 5.0 μg/kg and above resulted in some accumulation in the plasma. Accumulation was not consistently observed at the lower doses of 0.1 and 0.5 μg/kg.

EXAMPLE 9

A 28 Day Oral Toxicity Study in Dogs with Semi-Solid #3

[0106] In this study a 28-day repeated dose toxicology study of semi-solid #3 was conducted in dogs to assess the pharmacokinetics of calcitriol after weekly oral capsule dosing. Semi-solid #3 or control article capsules were administered on study days 0, 7, 14, 21, and 28. Twelve dogs (6 male, 6 female) received vehicle control (group 1), eight dogs (4 male, 4 female) received 0.1 μg/kg semi-solid #3 (group 2), and eight dogs (4 male, 4 female) received 1.0 μg/kg semi-solid #3 (group 3). Twelve dogs (6 male, 6 female) received 30.0 μg/kg semi-solid #3 on day 0 (group 4). Due to the severity of the clinical response observed after the first 30 μg/kg dose on day 0, dose levels were reduced in this group to 10 μg/kg (males on days 7, 14, 21, and 28) or 5 μg/kg (females on days 7, 14, 21, and 28). Blood samples were collected on each dog pre-dose and at 1, 2,4, 6, 8, 24, and 48 hours following dosing on study days 0 (first dose) and 21 (fourth weekly dose). All animals were sacrificed on study day 29.

[0107] The pharmacokinetic results for plasma calcitriol for groups 2-4 are summarized in Table 16.

Table 16: Mean Toxicokinetic Parameters of Calcitriol After Weekly Dosing with Semi-Solid #3 in Dogs

DAY 0						
Dose	0.1 µg/kg (Group 2)		1.0 µg/kg (Group 3)		30.0 µg/kg (Group 4)	
Sex (No. of Dogs)	Male (4)	Female (4)	Male (4)	Female (4)	Male (6)	Female (6)
C_{max}, pg/mL	198.7	430.8	2385.0	3419.1	84909.1	57133.3
T_{max}[a], h	1.0	2.0	1.0	1.5	2.0	2.0
AUC_{0-24}, pg·hr/mL	1840.6	3093.4	17144.2	23259.7	496044.6	323573.1
AUC_{0-48}, pg·hr/mL	2130.8	3093.4	19141.6	25794.5	644064.2	365340.7

DAY 24 (Fourth Weekly Dose)						
Dose	0.1 µg/kg (Group 2)		1.0 µg/kg (Group 3)		10.0 µg/kg (Group 4)	5.0 µg/kg (Group 4)
Sex (No. of Dogs)	Male (4)	Female (4)	Male (4)	Female (4)	Male (6)	Female (6)
Dose	0.1	0.1	1.0	1.0	10.0[b]	5.0[b]
C_{max}, pg/mL	217.6	398.3	2272.1	2188.6	29061.8	8670.7
T_{max}[a], h	1.0	2.0	1.5	2.0	1.0	2.0
AUC_{0-24}, pg·hr/mL	1956.2	3283.0	19765.4	12947.3	173597.2	46878.1
AUC_{0-48}, pg·hr/mL	2225.9	3640.7	24606.9	15380.0	209732.1	54976.1

[a]The values for T_{max} are the median values for this parameter. All other parameters shown are mean values.
[b]Doses of semi-solid #3 were lowered beginning on Study Day 7.
Data from the vehicle control dogs (Group 1) were not subjected to pharmacokinetic analysis.

[0108] Figs. 3A and 3B show the adjusted plasma concentration-time curve for calcitriol after oral capsule dosing with semi-solid #3 on study days 0 and 21 in male (Fig. 3A) and female (Fig. 3B) Beagle dogs. Calcitriol values at time 0 on day 0 were subtracted from all subsequent timepoints to adjust for endogenous (baseline) plasma calcitriol

[0109] The results of the study indicate that following:

- After oral capsule dosing with semi-solid #3, plasma concentrations of calcitriol rose fairly rapidly, reaching peak plasma concentrations within two hours.
- Plasma concentrations of calcitriol decreased at a more rapid rate during the first 8 hours post-dosing than during the later timepoints (24-48 hours), possibly indicating redistribution of calcitriol to extravascular spaces, with subsequent slow release of calcitriol back into the vascular spaces. This observation was more apparent at the higher dose levels than at the lower dose levels.
- At 24 hours post-dosing, plasma concentration of calcitriol had declined to near-baseline values at the low dose of 0.1 μg/kg. However, at the higher doses of calcitriol, dose-related residual concentrations of calcitriol were still evident at the last sampling timepoint (48 hours), although all values returned to pre-dose (baseline) values by one week post-dosing.
- Values for C_{max} and AUC were fairly proportional to dose throughout the dose range tested (0.1-30.0 μg/kg).
- Values for AUC_{0-24} at the low dose, which was the no observable adverse effect level (0.1 μg/kg) ranged from 1840.6 - 3283.0 pg·hr/mL.
- Values for AUC_{0-24} at the mid dose, which was the maximum tolerated dose (1.0 μg/kg) ranged from 12,947.3 – 23,259.7 pg·hr/mL.
- Values for AUC_{0-24} at doses associated with weight loss and moderate signs of toxicity, ranged from 46,878.1 pg·hr/mL(5.0 μg/kg; females) to 173,597.2 pg·hr/mL (10.0 μg/kg; males).
- Values for AUC_{0-24} at a dose associated with mortality (30.0 μg/kg) ranged from 323,573.1 – 496,044.6 pg·hr/mL.
- There were no consistent sex differences in any pharmacokinetic parameter.

[0110] Overall, the animals appeared to handle calcitriol similarly after the first dose and after repeated once-weekly dosing, with a few exceptions such as higher values for C_{max} and AUC on Day 0 compared to Day 21 in the 1.0 μg/kg females (not evident in the males).

EXAMPLE 10

Acute Toxicity Study of Three Different Formulations

[0111] In the study described in Example 7, several in-life parameters, including clinical chemistry parameters, were monitored to assess the toxicity of the calcitriol formulations. Blood samples were analyzed for calcium, phosphorus, blood urea nitrogen (BUN), glucose, albumin, bilirubin (total), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), and creatinine.

[0112] No clinical toxicity was seen in any dog with any of the three formulations.

[0113] Hypercalcemia was seen after dosing with 1.0 µg/kg with all three formulations. The group mean and the individual range of serum calcium levels of each of the three different formulations are presented in Table 17.

Table 17: Group Mean Serum Calcium Levels (mg/dL)

Historical Control		ROCALTROL, 1.0 µg/kg						
		0 hr	4 hr	24 hr	48 hr	72 hr	96 hr	120 hr
9.25-11.3[a] (10.44)[b]	Mean	11.1	NA	13.8*	12.9*	NA	NA	NA
	SD	0.31	NA	0.83	0.26	NA	NA	NA
	Range	10.8-11.5	NA	13.2-15.0	12.6-13.1	NA	NA	NA
		Calcitriol, liquid, 1.0 µg/kg						
9.25-11.3 (10.44)	Mean	10.4	10.5	16.1*	14.3*	12.7*	12.5*	12.0*
	SD	0.17	0.37	1.47	1.34	0.53	0.78	0.80
	Range	10.2-10.5	10.1-10.9	13.9-17.0	12.9-15.7	12.0-13.3	11.5-13.4	11.2-13.1
		Calcitriol, semi-solid, 1.0 µg/kg						
9.25-11.3 (10.44)	Mean	10.1	10.6	14.3*	14.2*	12.3*	12.6*	12.7*
	SD	0.33	0.29	1.72	1.52	1.35	0.76	0.47
	Range	9.7-10.5	10.7-10.8	12.2-16.4	12.1-15.5	10.8-13.6	11.5-13.1	12.0-13.0

[a] Historical range
[b] Historical mean
* Mean outside historical range
NA = not available (serum sample not taken)

[0114] In addition to elevations of calcium, elevations of ALT, AST, BUN, and creatinine were observed in all groups.

[0115] In summary, the results of this study indicated that:

- No treatment-related clinical signs were evident in any dog after dosing with any of the formulations (ROCALTROL, liquid, or semi-solid).
- Hypercalcemia at 1.0 µg/kg PO was seen in dogs with all three formulations.
- Time course of the hypercalcemia was comparable among all three formulations up to 48 hours; sampling for the ROCALTROL group did not extend beyond 48 hours.
- Severity of the hypercalcemia was comparable among the three formulations; the highest serum calcium (17.0 mg/dL) occurred at 24 hours in dogs receiving the liquid formulation.
- Mean values for ALT, AST, BUN, and creatinine were observed to be outside the historical range in all treatment groups at one or more timepoints.
- Elevations for BUN and creatinine were greater in the liquid or semi-solid groups; in the absence of a concurrent control group, the significance of this observation is unclear.

EXAMPLE 11

Acute Maximum Tolerated Dose Study

[0116] In the study described above in Example 8, the acute toxicity and hypercalcemia effects of semi-solid #3 were also assessed to estimate the maximum tolerated dose and to provide data for dose selection of future studies.

[0117] Calcium levels were increased in a dose-related manner at all dose levels in males (Fig. 4A) and females (Fig. 4B). Serum calcium data for the 0.001 and 1.0 µg/kg dose was obtained in male dogs in the study describe in Example 10, and is included here for completeness.

[0118] In summary, this study of semi-solid #3 administered orally via a capsule to male and female Beagle dogs at 0.1, 0.5, 5.0, 10.0, 30.0, and 100.0 µg/kg showed:

- Dose dependent hypercalcemia was the most common laboratory abnormality.
- Elevations of creatinine, urea nitrogen, cholesterol, erythrocytes, hemoglobin, hematocrit, and neutrophils, and a decrease in lymphocytes were seen at doses of 5.0 µg/kg or higher.
- Body weights and food consumption decreased markedly after receiving the 30.0 and 100.0 µg/kg doses; after 100.0 µg/kg, dogs had a noticeable thin appearance and obvious decreased activity.

[0119] Based on these results, the maximum tolerated dose of semi-solid #3 in dogs appeared to be 5.0 µg/kg.

EXAMPLE 12

A 28 Day Repeated Dose Toxicity Study

[0120] In the study described above in Example 9, the dogs were also assessed for potential toxicity of the semi-solid #3 formulation when administered to dogs by the oral (capsule) route once every seven days for 28 days. The study included assessments of clinical signs, body weights, food consumption, toxicokinetics, clinical pathology including biochemistry, hematology, coagulation, and urinalysis, ophthalmology, cardiology, gross necropsy, organ weight, and full histopathology on all animals. The study design is summarized in Table 18.